Aerosol Stability of Bovine Adenovirus Type 3

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ABSTRACT

The WBR-1 strain of bovine adenovirus type 3 was suspended in Eagle's medium or bovine nasal secretion and atomized into a rotating drum at temperatures of 6°C or 32°C and relative humidities of 30% or 90%. Impinger samples of the aerosols were collected seven minutes, one, two and three hours postgeneration, and titrated for infectivity in embryonic bovine kidney cell cultures. Under certain conditions of temperature and relative humidity, the virus was more stable in aerosols of Eagle's medium than in nasal secretion. The bovine adenovirus was usually inactivated more rapidly at 30% relative humidity than at 90% relative humidity and during aging of the aerosols the virus was inactivated more rapidly at 32°C than at 6°C.

RÉSUMÉ

Cette expérience visait à produire des aérosols contenant la souche WBR-1 du type 3 de l'adénovirus bovin, avec un pulvérisateur Devilbiss 40, à partir du milieu essentiel minimum d'Eagle et des sécrétions nasales d'un veau témoin; on entreposa ensuite ces aérosols, dans un cylindre rotatif, à la température de 6° ou 32°C, combinée à une humidité relative de 30% ou de 90%. On préleva des échantillons de ces aérosols, avec un appareil en verre (AGI-30), aux intervalles suivants: sept minutes, une, deux et trois heures après la pulvérisation; on titra ensuite leur infectivité, à l'aide de cultures de cellules rénales de foetus bovins. Dans certaines conditions de température et d'humidité relative, le virus se révéla plus stable dans les aérosols du milieu d'Eagle que dans ceux des sécrétions nasales du veau témoin. L'inactivation du virus s'avéra plus rapide à une humidité relative de 30% que de 90%. Au cours du vieillissement des aérosols, l'inactivation du virus s'avéra plus rapide à 32°C qu'à 6°C.

INTRODUCTION

Bovine adenovirus type 3 (BAV-3), first isolated in England (4) has subsequently been established as a cause of respiratory disease in calves (6, 15). There is some evidence that respiratory diseases caused by bovine adenoviruses may be more common in the late winter and early spring (5). This higher incidence could be associated with climatic conditions which might favour the airborne spread of the infection. The literature on the survival of airborne pathogens of farm animals was recently reviewed (9) but reference was not made to bovine adenoviruses in

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this context, although the effect of relative humidity (RH) on the survival of human adenovirus has been studied by several workers (3, 7, 16). No data have been published on the effects of tempeature on aerosols of adenoviruses.

The present study was designed to provide information on the effects of temperature and RH on the survival of BAV-3 in aerosols generated from Eagle's medium and from bovine nasal secretion. In previous papers we have reported similar studies with infectious bovine rhinotracheitis (13) and parainfluenza virus type 3 (14).

MATERIALS AND METHODS

Cultivation and Assay of Bovine Adenovirus Type 3

The WBR-1 strain (4) of BAV-3 was cultivated in suspended MDBK cells (American Type Culture Collection) as previously described (12). The infectivity of the clarified supernatant stock virus was $10^{7.5}$ median tissue culture infectious doses ($TCID_{50}$) per ml. This stock virus was concentrated to a titre of 10^{10} $TCID_{50}$ per ml by ultracentrifugation at 73,368 g for three hours in the type 30 rotor in the Beckman model L-2 ultracentrifuge.

Infectivity titrations on the above virus preparations, on the virus suspensions which were nebulized and on the impinger fluids which were used to sample the aerosols were performed on monolayer cultures of the second passage of embryonic bovine lung cells in Linbro microtitre plates.[1] The growth medium for the cell cultures was minimum essential medium with Hank's salts[2] supplemented with 10% fetal bovine serum (FBS) and antibiotics (250 iu/ml penicillin and 100 μg/ml streptomycin. For maintenance, Eagle's minimum essential medium (EMEM) was supplemented with 5% FBS and antibiotics and buffered with HEPES at a concentration of 0.045 M. Tenfold dilutions of the samples to be assayed for infectivity were prepared in maintenance medium. Each dilution was inoculated in volumes of 0.1 ml into eight wells in the microtitre plate. The infectivity titre of the sample was then calculated by the

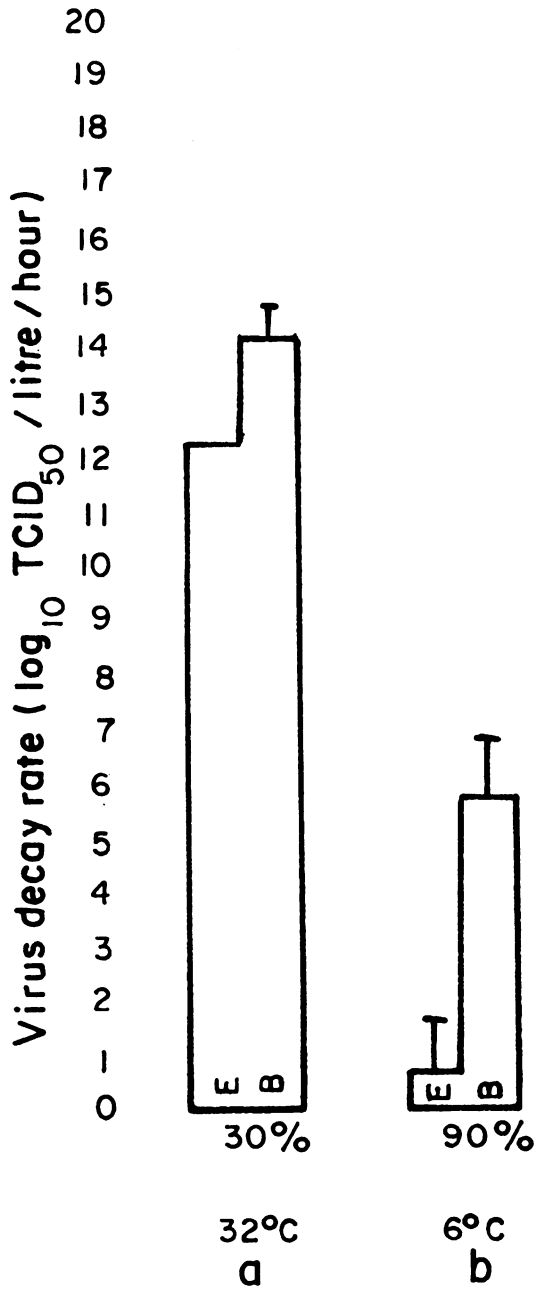

Fig. 1a, b. Effect of nasal secretion from noninfected calf (B) and Eagles minimum essential medium (E) on the decay rate of bovine adenovirus type 3 in aerosols during the spray period at different temperatures (32°C, 6°C) and RH (30%, 90%).

[1]Flow Laboratories, Mississauga, Ontario.

[2]Grand Island Biological Company, Burlington, Ontario.

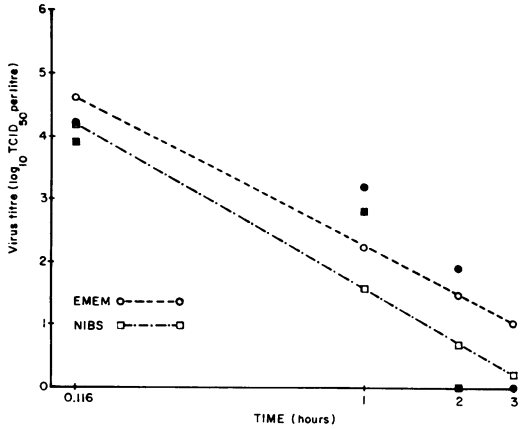

Fig. 2a. Effect of medium on the biological decay of bovine adenovirus type 3 in aerosols at 32°C and 30% RH during the aging period. ○ □ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = nasal secretion from noninfected calf.

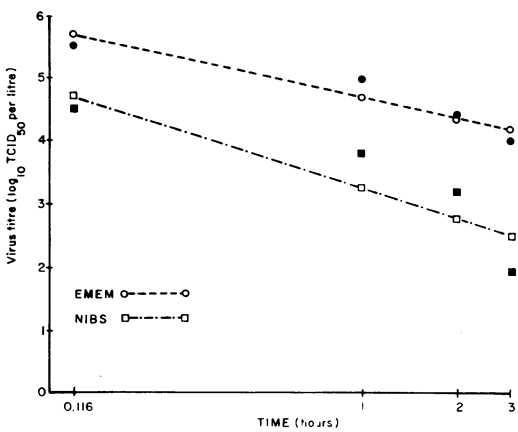

Fig. 2b. Effect of medium on the biological decay of bovine adenovirus type 3 in aerosols at 32°C and 90% RH during the aging period. ○ □ = average of estimated virus ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = nasal secretion from noninfected calf.

Spearman-Kaerber procedure on the basis of characteristic cytopathic effects.

Aerobiological Procedures

Bovine adenovirus type 3, concentrated as described above, was diluted 10^{-2} in EMEM or nasal secretion from a noninfected calf (NIBS) which was collected as described (17) from a colostrum-deprived calf which lacked antibodies

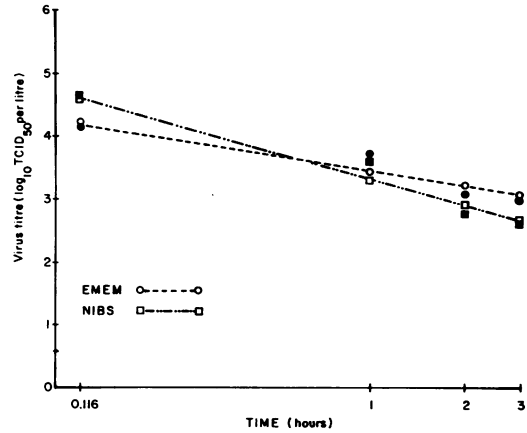

Fig. 2c. Effect of medium on biological decay of bovine adenovirus type 3 in aerosols at 6°C and 30% RH during the aging period ○ □ = average of estimated virus titre. ● ■ = average of observed virus titre. EMEM = Eagle's minimum essential medium. NIBS = nasal secretion from noninfected calf.

against BAV-3 and stored at –70°C. The virus suspensions were atomized with a Devilbiss 40 nebulizer for five minutes into a 200 litre drum rotating at 3 rpm (18). This was followed by a stabilization period of two minutes, after which the first aerosol sample was collected (seven minutes after the start of nebulization) and a further aging period of three hours during which the aerosol was sampled at hourly intervals. The aerosols were sampled with an all glass impinger (AGI-30) as described previously (13). Aerosols were generated from both of the above media at temperatures of 6°C and 32°C and at 30% and 90% RH and three replicate experiments were performed for each set of conditions. The detailed aerobiological procedures, including the determination of the particle size and physical decay of the aerosols, and the sampling protocol and medium were identical to those described previously (13).

Calculation and Analysis of Biological Decay Rates

The decrease in virus concentration due to loss of viral infectivity in the air (biological decay) was expressed as the biological decay rate. The biological decay rate (V) during the first seven minutes after the beginning of nebulization of the virus (spray period) was computed accord-

ing to the formula $V = \frac{\log No - \log Nt}{t}$, in which log No represents the virus concentration in the aerosol at time O, calculated from the volume and titre of the suspension which was nebulized, log Nt is the concentration of virus recovered at seven minutes and t is time in hours (0.116 h). The biological decay rate (regression coefficient) between seven minutes and three hours postgeneration (aging period) was calculated by the method of least squares (19). The biological decay rates obtained for aerosols of EMEM and NIBS during the spray and aging periods were subjected to analysis of variance (19) to compare the effect on decay rate of suspending medium at each temperature and humidity combination, of temperature within the same medium and RH, and of RH within the same medium and temperature. A significance level of 1% was used. When the results of these comparisons were significantly different during the aging period, virus inactivation curves (regression lines) were plotted for each comparison. The regression equation (19) used to calculate these virus inactivation curves was $y = a + \log x$, where y is the expected virus concentration in log $TCID_{50}$ per litre, a is the point where the line crosses the y-axis (y-intercept), b is the average decay rate (re-

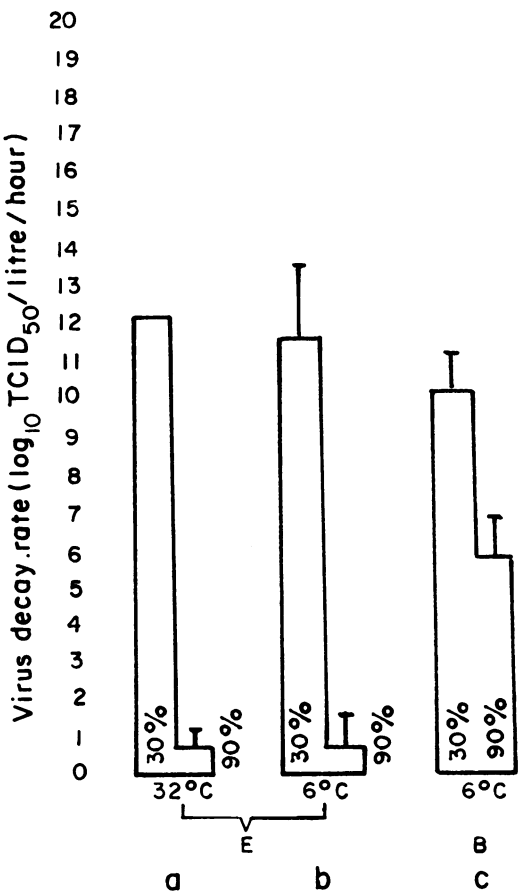

Fig. 3a, b, c. Effect of RH (30%, 90%) on the decay rate of bovine adenovirus type 3 in the aerosols of Eagle's minimum essential medium (E) and of nasal secretion from noninfected calf (B) at different temperatures (32°C, 6°C).

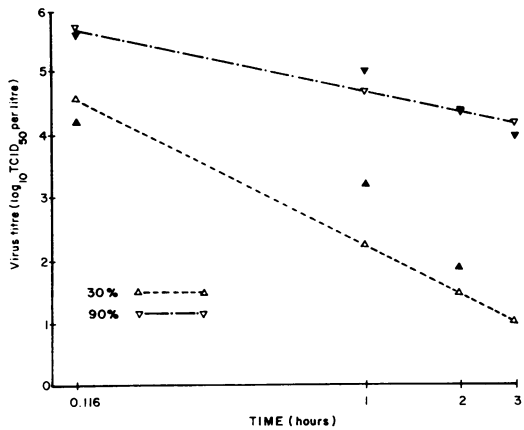

Fig. 4a. Effect of RH (%) on the biological decay of bovine adenovirus type 3 in aerosols of EMEM at 32°C during the aging period. ▽△ = average of estimated virus titre. ▼▲ = average of observed virus titre.

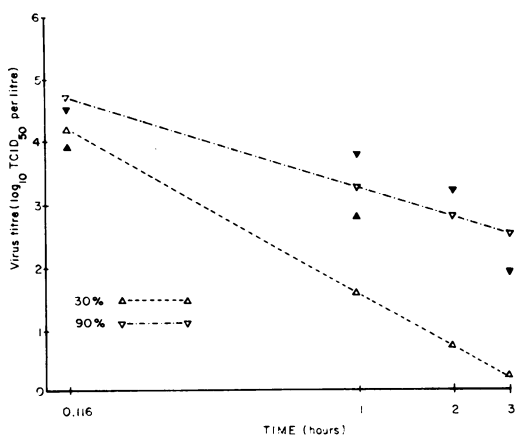

Fig. 4b. Effect of RH (%) on the biological decay of bovine adenovirus type 3 in aerosols of nasal secretion from noninfected calf at 32°C during the aging period. ▽△ = average of estimated virus titre ▼▲ = average of observed virus titre.

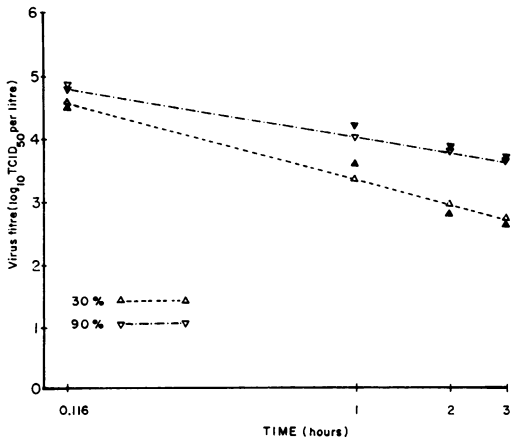

Fig. 4c. Effect of RH (%) on the biological decay of bovine adenovirus type 3 in aerosols of nasal secretion from noninfected calf at 6°C during the aging period. ▽△= average of estimated virus titre. ▼▲= average of observed virus titre.

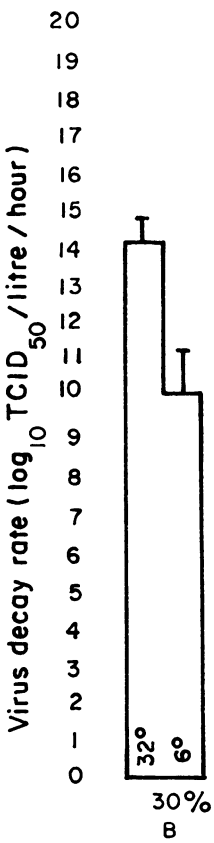

Fig. 5. Effect of temperature (32°C, 6°C) on the decay rate of bovine adenovirus type 3 in aerosols of nasal secretion from noninfected calf (B) at 30% RH.

gression coefficient or slope of the line) based on three replicates, and log x is the log_{10} of time in h, commencing seven minutes postnebulization.

RESULTS

The infectivity titres of BAV-3 in the initial aerosols and the titres subsequently recovered at seven minutes, one, two and three hours postgeneration from aerosols of EMEM and NIBS at different temperatures are recorded in Table I. Analysis of the biological decay rates revealed that the virus was less stable in aerosols of NIBS than in aerosols of EMEM during the spray period at 32°C and 30% RH (Fig. 1a) and at 6°C and 90% RH (Fig. 1b), as well as during the aging period at 32°C and both levels of RH (Fig. 2a, b) and at 6°C and 30% RH (Fig. 2c). Furthermore, BAV-3 was less stable in aerosols at the lower level of RH (30%) than at 90% RH in both media and at both temperatures (Fig. 3a, b, c; 4a, b, c) except for aerosols of NIBS at 32°C during the spray period, and during aging of aerosols of EMEM at 6°C, when changes in RH did not significantly influence the biological decay rate of the virus. During the spray period, temperature had no significant effect on the biological decay rate of the virus except in aerosols of NIBS at 30% RH, in which inactivation was more rapid at 32°C than at 6°C (Fig. 5), but during the aging period the virus was always inactivated more rapidly at the higher temperature, irrespective of the medium or RH (Fig. 6a, b, c, d).

DISCUSSION

Although BAV-3 was somewhat less stable in NIBS than in EMEM, it survived sufficiently well in aerosols of NIBS to suggest the possibility of airborne transmission of the virus under field conditions. The survival of the virus in aerosols was markedly enhanced by low temperature and high RH, and in the present experiments an aerosol stored for two hours at 6°C and 90% RH contained almost 3000 times more infectivity than a similar aerosol held at 32°C and 30% RH.

TABLE I. Recovery of Bovine Adenovirus Type 3 from Aerosols of Nasal Secretion from Noninfected Calf and Eagle's Minimum Essential Medium (EMEM) at Different Temperatures (T) and Relative Humidities (RH) During Spray and Aging

		Mean Virus Titre (Log_{10} $TCID_{50}$/Litre) ± SD									
		EMEM					BNSI				
T	RH	0	7 min	1 h	2 h	3 h	0	7 min	1 h	2 h	3 h
32°C	30%	5.6 ±0.21	4.2 ±0.21	3.2 ±0.12	1.9 ±0.17	0.0 ±0.00	5.6 ±0.25	3.9 ±0.25	2.8 ±0.23	0.0 ±0.00	0.0 ±0.00
	90%	5.7 ±0.15	5.6 ±0.10	5.0 ±0.30	4.4 ±0.20	4.0 ±0.12	5.6 ±0.12	4.5 ±0.53	3.8 ±0.59	3.2 ±0.06	1.9 ±0.00
6°C	30%	5.5 ±0.12	4.1 ±0.15	3.7 ±0.25	3.1 ±0.17	3.0 ±0.12	5.8 ±0.06	4.6 ±0.10	3.6 ±0.25	2.8 ±0.26	2.6 ±0.21
	90%	5.7 ±0.12	5.7 ±0.23	5.3 ±0.12	4.9 ±0.06	4.6 ±0.20	5.5 ±0.06	4.8 ±0.00	4.2 ±0.30	3.8 ±0.10	3.6 ±0.12

SD = standard deviation

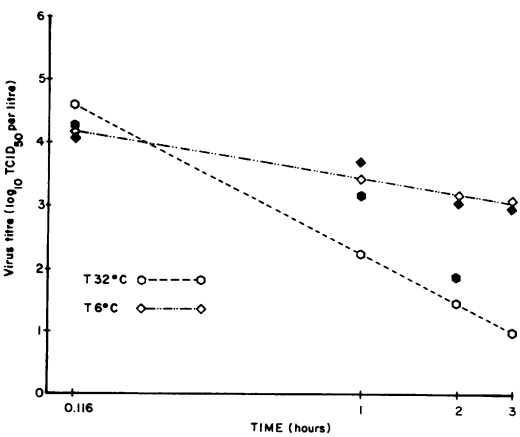

Fig. 6a. Effect of temperature (T) on the biological decay of bovine adenovirus type 3 in aerosols of Eagle's minimum essential medium at 30% RH during the aging period. ○ ◇ = average of estimated virus titre. ● ◆ = average of observed virus titre.

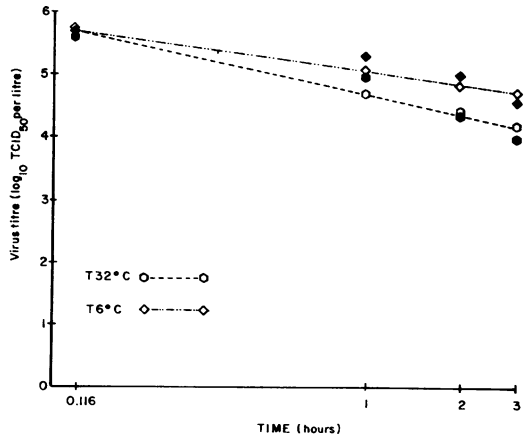

Fig. 6b. Effect of temperature (T) on the biological decay of bovine adenovirus type 3 in aerosols of Eagle's minimum essential medium at 90% RH during the aging period ○ ◇ = average of estimated virus titre. ● ◆ = average of observed virus titre.

This finding may be of considerable epidemiological significance in relation to airborne spread of the virus and may be related to the reported seasonal incidence of bovine adenovirus infections (5).

While no data are available in the literature on the effects of RH on the stability of airborne bovine adenoviruses, our finding that BAV-3 was more stable at the higher level of RH is in accord with studies (16) on aerosols of human adenovirus type 7 in Eagle's medium at room temperature and with observations that many nonenveloped viruses (2), with certain exceptions (8), survive best at high RH. It is of interest that bovine adenovirus type 1 was found to be resistant to surface inactivation, while several enveloped viruses which are unstable in aerosols at high RH were susceptible to surface inactivation (10).

There is evidence from the literature (9) that the survival of aerosols of certain viruses is favoured by lower temperatures but no data are available on the temperature dependence of the infectivity of aerosols of adenovirus. Our finding that the effect of temperature on the survival of BAV-3 was evident mainly during the aging period supports the suggestion (1) that while the inactivation of viruses immediately after aerosol forma-

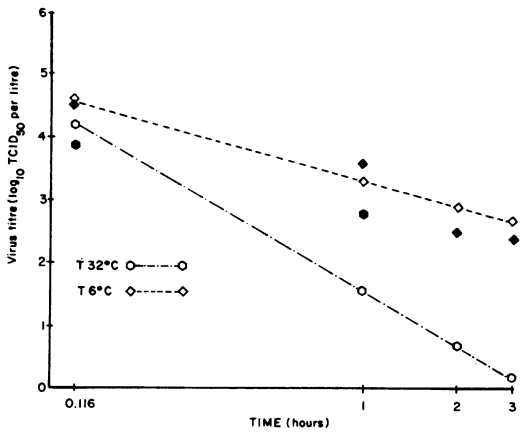

Fig. 6c. Effect of temperature (T) on the biological decay of bovine adenovirus type 3 in aerosols of nasal secretion from noninfected calf at 30% RH during the aging period ○ ◇= average of estimated virus titre. ● ◆ = average of observed virus titre.

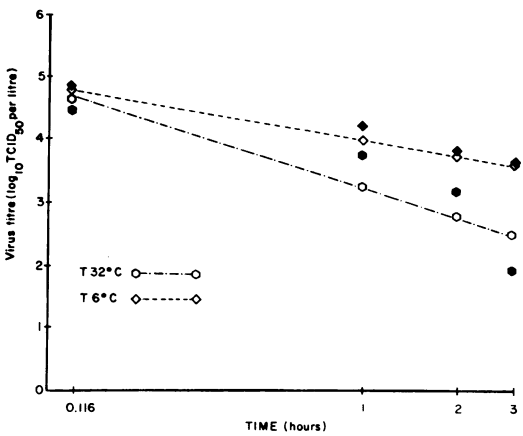

Fig. 6d. Effect of temperature (T) on the biological decay of bovine adenovirus type 3 in aerosols of nasal secretion from noninfected calf at 90% RH during the aging period. ○ ◇ = average of estimated virus titre ● ◆ = average of observed virus titre.

tion is RH-dependent, temperature effects may occur secondarily, and emphasizes the need for observations on the aging of aerosols in addition to experiments which are restricted to the immediate postgeneration period.

The lower biological decay rates for BAV-3 in aerosols of EMEM compared with NIBS cannot be fully explained at this time, although it is well recognized that the composition of the suspending fluids plays an important role in virus stability in the air. Previous studies (11) indicated that the particle sizes of aerosols generated from EMEM and NIBS were not widely different but that the glucose and amino acid content of EMEM might be responsible for its ability to protect viral aerosols against inactivation. Glucose appeared to provide the higher level of protection during spraying and amino acids seemed to be more protective during aging.

The present findings with BAV-3 suggest that a relatively high temperature and low RH might reduce the risk of airborne transmission of bovine adenovirus among housed calves. Thus, manipulation of the environment might be of value in the control of this infection. However, since our previous studies showed that infectious bovine rhinotracheitis virus (13) and parainfluenza virus type 3 (14) were inactivated most rapidly at a temperature of 32°C and 90% RH, spread of these infections might be reduced at high rather than low RH, although for these viruses as well as BAV-3, warmth might be an important factor in reducing transmission. Further studies of the aerosol stability of bovine respiratory viruses at other temperature and RH levels might provide a better basis for attempted control by environmental manipulation. It would also be necessary to consider the possible effects of temperature and RH on the susceptibility of calves to infection.

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